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Title: Determination of Cadmium and Lead by ICP-MS		
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Contents

A. INTRODUCTION

1. Theory

   Sample tissue is digested with concentrated nitric acid in a microwave digestion apparatus. The resulting sample digest is diluted, fortified with internal standards, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

2. Applicability

   This method is applicable to the analysis of cadmium (Cd) and lead (Pb) at ppb levels in beef, pork and poultry muscle, liver, and kidney.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

   a. Analytical balance - sensitive to 0.1 mg.

   b. Microwave digestion system - Mars5 System, CEM.

   c. Microwave digestion vessel - high pressure, 100 ml capacity. Model XP-1500, with TFM liners, CEM.

   d. Vacuum Concentration/Drying apparatus - Microvap accessory set for Mars5 system, CEM.

   e. Stirring rods - Teflon or polypropylene.

   f. Volumetric flasks - polypropylene or polymethylpentane, 50 and 100 mL.

   g. Volumetric flasks - glass, 10 - 1000 mL, as needed for preparation of standards, reagents.

   h. Micropipettors - fixed or variable, covering ranges 10 -1000 µL.

   i. Bottles - polypropylene, 100 and 250 mL.

   j. Centrifuge tubes - polypropylene, 15 and 50 mL.

   k. Argon gas, high purity grade (99.99%).

   l. Syringe filter - Acrodisc CR 13 mm, with 0.2 um PTFE Membrane, Gelman Laboratory.

2. Instrumentation

   Inductively Coupled Plasma Mass Spectrometer - Agilent model 7500a.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents/solutions may be substituted.

1. Reagents

   a. Millipore water - Deionized water polished to ASTM CAP/NCCLS Type 1 specifications or better (resistance ≥ 18 megohms).

   b. Nitric acid - concentrated, ultra-pure, stored in Teflon. Optima by Fisher or Double Distilled by GFS.

   c. Sodium Hydroxide - reagent grade.

2. Solutions

   a. 25% NaOH solution (for evaporation scrubber):

      Weigh 250 grams of NaOH into a 1 L volumetric flask and bring up to volume with water.

   b. 1% and 2% HNO_3 solutions:

      Dilute ultra-pure nitric acid 1:100 and 1:50, respectively, with Millipore water. Prepare and store in polypropylene bottles.

D. STANDARDS

Note: All elemental standard and internal standard solutions are prepared from commercially available reference standard materials. Standard solutions must be ICP-MS grade.

1. Source

   a. Elemental standard solutions are available from:

      i. SPEX CertiPrep 203 Norcross Avenue, Metuchen, NJ 08840.

      ii. Inorganic Ventures, Inc. 195 Lehigh Ave, Suite 4, Lakewood, NJ · 08701

      iii. Mass spectrometer tuning solution is available from Agilent Technologies

2. Preparation of Standard Solutions

   Important: Metals may adsorb onto glass surfaces. Store all solutions in polypropylene or other inert containers.

   a. Internal standard (ISTD), Indium and Terbium, 5000 µg/L

      Add 50 mL each of 10,000 µg/L indium and terbium standards to a 100 mL polypropylene bottle.

Note: Other internal standards can be used as long as the element is not contained in the sample, the mass number is close to that of the analyte, and the ionization potential is close to that of the analyte.

b. Calibration Standards

Prepare calibration standards for constructing a multipoint standard curve covering the range of analyte concentrations anticipated in samples.

Prepare intermediate standards by making dilutions of commercially available 1000 mg/L standard solutions into 2% HNO_3. Suggested concentrations are:

i. 10,000 µg/L - Pipet 100 µL of 1000 mg/L standard to a 10 mL volumetric flask and dilute to volume.

ii. 1000 µg/L - Pipet 100 µL of 1000 mg/L standard to a 100 mL volumetric flask and dilute to volume.

iii. 100 µg/L - Pipet 1000 µL of 10,000 µg/L solution to a 100 mL volumetric flask and dilute to volume.

Prepare calibration standards by making appropriate dilutions of intermediate standards with 2% HNO_3 and adding sufficient 5000 µg/L ISTD to result in a final ISTD concentration of 5 µg/L. Prepare these standards using polymeric volumetric flasks.

The Table below lists some suggested concentrations for calibration standards and recommended volumes and concentrations of solutions required for preparation of 100 mL volumes of each.

Calibration STD [Sample Equivalent* in ( )]	Amount used x Intermediate Standard concentration	Amount ISTD
Calibration Blank (0 ppb)	(2% HNO_3 Only)	100 µL
0.05 µg/L (5 ppb)	50 µL x 100 µg/L	100 µL
0.10 µg/L (10 ppb)	100 µL x 100 µg/L	100 µL
0.20 µg/L (20 ppb)	200 µL x 100 µg/L	100 µL
0.50 µg/L (50 ppb)	500 µL x 100 µg/L	100 µL
1.00 µg/L (100 ppb)	100 µL x 1000 µg/L	100 µL
2.00 µg/L (200 ppb)	250 µL x 1000 µg/L	100 µL
5.00 µg/L (500 ppb)	500 µL x 1000 µg/L	100 µL
10.00 µg/L (1000 ppb)	1000 µL x 1000 µg/L	100 µL

*Equivalent Analyte concentration in a sample, assuming a sample concentration of 0.01 g/mL (0.5 g/50 mL) in final extract.

c. Quality Control Standards

Prepare Quality Control standards from commercially available 1000 mg/L standard solutions obtained from a *different source* than that used to prepare Calibration Standards. Two types of quality control standard must be prepared:

i. QC Standard:
Prepare a combined Pb/Cd standard having concentrations near the midpoint of the calibration curve, but different from those used in any calibration standard. Prepare in same manner calibration standards are prepared, diluting with 2% HNO_3 and adding sufficient 5000 µg/L ISTD to result in a final ISTD concentration of 5 µg/L.

ii. Fortification Standard:

Prepare a combined Pb/Cd standard suitable for preparation of positive controls by making quantitative dilutions of the 1000 mg/L standard into 2% HNO_3. Prepare solutions at a concentration sufficient to permit a fortification volume between 25 - 250 µL to be used. A 500 µg/L fortification standard, for example, would be applicable over a 25-250 ppb range, based on a nominal sample weight or 0.5 g.

d. Mass Spectrometer Tuning Solution

This is a commercially available 10 ng/mL solution of Lithium, Yttrium, Cerium, Thallium, and Cobalt in 2% HNO_3. (Agilent Cat # 5184-3566).

3. Storage and Stability.

a. All standards may be stored at room temperature.

b. Commercially available standard solutions may be used until their expiration date.

c. Solutions made from these may be used up to the earliest expiration date of any standard used to prepare them.

E. SAMPLE PREPARATION

Note: Since trace amounts of lead and cadmium are ubiquitous in the environment and may be present in dust particles, efforts should be made to avoid external contamination. All areas involved in sample preparation and analysis should be kept as dust-free as possible to minimize the chance of sample or apparatus contamination.

Samples must be thoroughly blended to assure uniformity prior to removal of a test portion.

F. ANALYTICAL PROCEDURE

1. Microwave Digestion

   a. Weigh homogenized sample to nearest .01 g into a clean[1] microwave vessel liner. Use approximately 0.5 g for muscle tissues, 0.5 - 1 g for liver and kidney[2].

      Note: Prepare negative and positive controls at this time (See section I.5, "Sample Set"). Fortify positive control(s) using 25 - 250 µL of an appropriate Fortification Standard.

      [1]Vessel liners must be cleaned after each use to reduce the possibility of cross-contamination. Refer to Section K.2 for recommended cleaning procedure.

      [2]Caution! Mixing sample types or sample weights may produce unacceptably large variations in pressures developed during digestion, possibly resulting in damage to vessels if unvented caps are used. In order to maintain relatively constant digestion conditions in all vessels, analyst should attempt to digest like quantities of similar samples in each batch.

   b. Add 5mL of concentrated HNO_3.

   c. Assemble the vessel according to the manufacturer's instructions.

   d. Place assembled vessels into the microwave according to the manufacturer's instructions.

   e. Set microwave oven program as follows:

Power:	1200 Watts*
Ramp Time:	10 minutes
Final Temperature:	180 °C
Temperature Hold Time:	10 minutes
Cool down time:	10 minutes

      *If there are less than eight vessels in the microwave the wattage can be lowered to 600.

   f. Initiate oven program and digest samples.

   g. Allow vessels to cool, then transfer to a fume hood and allow coming to room temperature.

   h. Slowly open the vent fittings and vent to atmospheric pressure, then disassemble vessels.

2. Microwave Evaporation

   a. Place vessel liners into the evaporation carousel and assemble according to the

manufacturer's instructions.

b. Place the evaporation assembly into the microwave.

c. Set microwave oven program as follows:

Power:	600 Watts
Ramp Time:	5 minutes
Final Temperature:	120 °C
Temperature Hold Time:	3.5 minutes*
Cool down time:	10 minutes

*Typical value required when 8 vessels are used. Hold times required to achieve a final volume of 1 mL for any given number of vessels must be determined experimentally.

d. Initiate oven program and evaporate samples

e. Once vessels have cooled to room temperature, remove the evaporation assembly from the oven and dismantle.

3. Extract Preparation

a. If the solution volume remaining in the vessel liner is less than 1 mL, add concentrated HNO_3 to bring volume to approximately 1 mL. Note: residual acid volumes of up to 2.5 mL are acceptable, but should be avoided if possible. Pour extract solution into a 50 mL plastic tube containing approximately 10 mL Millipore water.

b. Quantitatively transfer residual digest by rinsing the liner 3 - 4 times with Millipore water, adding each rinse to the extract in the tube. Keep total rinse volume < 35 mL.

c. Add 50 µL of 5000 µg/L ISTD solution to the extract.

d. Bring extract volume to 50 mL with Millipore water.

e. Cap tube and invert several times to mix.

Note: The percentage of dissolved solids in the 50 mL extract, which is higher than that recommended by instrument manufacturer, can be reduced by increasing the dilution volume. Analyst must balance detrimental effects of high dissolved solids content (matrix effects, instrument contamination) against detrimental effects resulting from environmental contamination and lower analyte concentrations when considering this. If additional dilutions are made, care must be taken to maintain ISTD concentration at 5 ng/mL and adjust standard curve concentrations accordingly, if necessary.

4. ICP-MS Analysis

a. Tuning

i. Prior to sample analysis check the instrument's tuning parameters by analyzing the tuning solution as specified by the manufacturer. Check the sensitivity, % RSD, % oxide, % doubly charged, peak shape, and resolution.

ii. If these parameters are outside the manufacturer's specifications, retune the instrument.

b. ICP-MS Parameters

Set up instrument to monitor isotopes of cadmium and lead, indium and terbium (or other selected internal standards), and molybdenum (interferes with cadmium quantitation if present).

Table 1. ICP-MS Isotopes Monitored for Pb and Cd Analysis

Metal	Isotopes to Monitor
Cadmium	106, 108, 110, 111*, 112, 113, 114, 116
Lead	204, 206, 207, 208*
Molybdenum	92, 94, 95, 96, 97, 98, 100
Indium	113, 115
Terbium	159

* Isotope used for quantitation

c. Instrument calibration

i. Analyze a calibration blank followed by at least 4 calibration standards (D.2.b) covering the range of interest. Using linear regression analysis, plot relative response (response relative to ISTD response) vs. concentration in µg/L and determine slope (m), intercept (b), and correlation coefficient (r) of the calibration curve. Correlation coefficient must be ≥ 0.995, or calibration must be repeated.

ii. Analyze the calibration blank and a QC standard (D.2.c.i)immediately after the calibration curve. The value of the blank should be close to that initially recorded, and the concentration calculated for the QC standard must be within ± 10% of its accepted value. If these conditions are not met, the calibration sequence must be repeated until results are acceptable.

d. Sample Analysis

Once instrument meets calibration requirements, analyze all controls and test samples, taking care to meet conditions listed below.

i. Calibration blanks and QC standards must be included in the sample analysis sequence after at least every 12 consecutive samples analyzed, and also at the end of the sample sequence to verify instrument performance over the course of the run.

ii. If response of any sample exceeds highest standard in the calibration curve, make an appropriate dilution with a 5 µg/L solution of ISTD in 2% HNO_3 and re-analyze.

G. CALCULATIONS

Note: Instrument software can be programmed to perform all necessary calculations.

1. Using values for m, b determined for the calibration curve (F.4.c), determine analyte concentration (C_E, in ng/mL) in any extract having a relative response R using:

$$C_E \text{ (ng/mL)} = C_E, \mu g/L = (R-b)/m$$

Note: If sample is found to contain molybdenum, instrument software must be set to compensate for contribution of molybdenum oxide to the 111 ion used for quantitation of cadmium in the sample.

2. Calculate analyte concentrations in controls and samples (C_S) using:

$$C_S \text{ (ppb)} = \frac{C_E \times V \times D}{W}$$

Where:
C_E = Analyte Concentration in final extract, in ng/mL
V = Final extract volume in milliliters
D = Dilution factor (Diluted volume/aliquot volume), if secondary dilution made.
W = Sample Weight in grams.

3. Calculate Relative % Difference (RPD) for duplicate results using:

$$RPD = \frac{|C1 - C2| \times 100}{(C1 + C2)/2}$$

Where:
C1 = first duplicate's concentration.
C2 = second duplicate's concentration.

4. Calculate recoveries of fortified controls and check samples using

$$\% \text{ Rec} = \frac{(C_F - C_B) \times W \times 100}{V_{FS} \times C_{FS}}$$

Where
C_F, C_B = Analyte concentrations determined for the fortified sample and the blank tissue from which it was prepared, in ppb (ng/g).
W = Weight of fortified control, in grams.
V_{FS} = Volume of fortification standard added, in mL.
C_{FS} = Concentration of fortification standard, in ng/mL.

5. Acceptability Requirements:

Before results can be reported, the following quality control acceptability requirements must be met:

Each Sample batch must meet the following criteria:

a. The instrument calibration meets acceptance criteria specified in section F.4.c.

b. The recovery calculated for the set's positive control meets acceptance criteria specified in Section I.1.

c. If a positive control duplicate is run, the calculated RPD is ≤ 20%.

d. All calibration blanks injected show responses similar to those seen in previous blanks, and all QC standards calculate to be within ± 10% of their accepted value.

In addition, each test sample must meet the following criteria:

a. The sample internal standard areas must be within ± 20% of the average instrument calibration internal standard areas.

b. For a positive identification the analyte's isotope ratios must be correct. This may be determined by using Agilent software to visually compare relative ion abundances for all monitored isotopes against a plot of expected values. The analyte may be considered to be positively identified if ions from all monitored isotopes are present and at least 3 isotope ions are within approximately ± 15% of expected value after any necessary corrections for interferences have been applied.
Alternatively, identity may be confirmed by comparing response ratios of all monitored isotope ions (relative to the quantitation ion) in the sample against those of a reference standard. Analyte will be positively identified if all monitored ions are present and at least two of the calculated ion ratios are within 80 -120% of those calculated for a reference standard.

H. HAZARD ANALYSIS

1. Required Protective Equipment - Safety glasses, lab coat, protective gloves.

2. Hazards

Item/Procedure	Hazard	Recommended Safe Procedures
Nitric Acid	Strong oxidizer. May be fatal if swallowed or inhaled. Extremely corrosive. Contact with skin or eyes may cause severe burns and permanent damage.	Perform operations using concentrated acid in fume hood. Use protective eyewear, gloves and clothing. Store in approved acid safety cabinet away from basic or other reactive materials.
Microwave Digester	Possible explosion hazard	Follow manufacturer recommendations
Pb, Cd Standards	Poisonous if ingested.	Do not pipet by mouth

3. Disposal Procedures

Item/Procedure	Hazard	Recommended Safe Procedures
Nitric Acid	See above	Store in cabinet away from bases or other reactive materials. Follow applicable state and local regulations when disposing of acid solutions.
Pb, Cd Standards		Follow applicable state and local regulations when disposing of solutions or their residues.

I. QUALITY ASSURANCE PLAN

1. Performance Standard (FSIS requirements)

Analyte	*Analytical Range*	*Acceptable Recovery*
Pb, Cd	≥ 25 ppb (Pb) ≥ 10 ppb (Cd)	< 25 ppb: 70 – 110% ≥ 25 ppb: 80 – 110%

2. Readiness to Perform

   a. Familiarization

      i. Phase I: Standards - Analyze a ≥ 6 level standard curve in duplicate for Pb and Cd over range 0 – 5 µg/L (0r higher) on 3 different days. Suggested concentrations are:

         (a) 0 µg/L (calibration blank)

         (b) 0.2 µg/L

         (c) 0.5 µg/L

         (d) 1.0 µg/L

         (e) 2.0 µg/L

         (f) 5.0 µg/L

      ii. Phase II: Fortified samples - For muscle, liver, and kidney tissues: Fortify blank tissue in duplicate with Cd and Pb at 4 levels (including 0) representing low, medium, and high values in the range of interest for that tissue. Recommended ranges are muscle: up to100 ppb; liver and kidney: up to 250 ppb. Analyses must be conducted over a minimum of three separate days.

         NOTE: Phase I and Phase II may be performed concurrently.

      iii. Phase III: Check samples for analyst accreditation.

         (a) A minimum of six check samples, having concentrations unknown to the analyst. Set must include one blank tissue, with the remainder containing Pb at levels between 25 - 250 ppb and Cd at any levels between 10 - 250 ppb.

         (b) Approval from Quality Assurance Manager (QAM) is required to commence official analysis.

   b. Acceptability criteria.

      Refer to section I.1 above.

3. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency: One per week per analyst, when samples are analyzed.

      ii. Records are to be maintained by the analyst and reviewed by the supervisor and QAM.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

4. Sample Acceptability and Stability

a. Sample size: 450 g minimum.

b. Sample condition: Cold on receipt.

c. Sample storage:

i. Time: Indefinite.

ii. Condition: Frozen.

5. Sample Set

A sample set consists of:

a. One tissue blank (Negative control).
Note: Truly blank tissues are not available. Use previously analyzed tissues having low analyte levels for this purpose.

b. One or more fortified blanks (Positive controls), prepared using the same matrix used for the tissue blank.
Note: Use fortification levels greater than the amount naturally present in the blank to minimize the blank's contribution to the uncertainty of the calculated recovery.

c. As many samples as can be digested simultaneously with the positive and negative controls in the microwave apparatus.

6. Method Sensitivity

a. Lowest detectable level (LDL): Not Determined.*

b. Lowest reliable quantitation (LRQ): Not Determined.*

c. Minimum proficiency level (MPL): Pb: 25 ppb; Cd: 10 ppb.

* Background levels measured in tissues were too high or variable to allow accurate determination.

J. WORKSHEET

Not Included.

K. APPENDIX

1. References

Agilent 7500 ICP-MS Hardware Manual, G1833-90004, January 2001.

CEM XP-1500 Plus Vessel Accessory Sets and Autovent Option Instruction for Use, 600493, Rev. 5, 8/01.

CEM Vacuum Concentration/Drying Accessory Set Instructions for Assembly and Use, 600484, Rev. 1, 6/99.

EPA Method 6020, Inductively Coupled Plasma-Mass Spectrometry, Revision 0, September 1994.

2. Cleaning Vessel Liners

The following procedure was shown to be adequate for removal of residual adsorbed Cd and Pb from Teflon liners used in this method. Other procedures are available and may be used if shown to be effective.

a. Add approximately 20 ml of 2 % HNO3 to each digestion vessel liner.

b. Assemble vessels as specified by manufacturer.

c. Place in microwave.

d. Digest at 600W, Ramp to 125 °C over 10 minutes, then hold at temperature for 10 minutes.

e. Cool vessels to room temperature, then disassemble.

f. Rinse vessel liners and caps with Millipore water several times to remove all traces of acid.

g. Place in a clean environment to dry.

APPROVALS

Approval Records are on file.

Approved by	Date Approved
Eric Flynn	9/10/2003
Gina McLeroy	9/10/2003
Bill Koscinski	9/02/2003
Jess Rajan	9/08/2003
Charles Pixley	9/08/2003
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